the luciferase reporter gene. This transactivation was strongly attenuated by truncation of the gene from the C-terminal end. Further, the relative transactivation of the luciferase reporter by the full length RBT1 gave values higher than the positive control construct, TLS, in cell lines 293, MDA231, MCF7 and Saos-2, suggesting a possible role in cancer.

RBT1 binds RPA32 in the yeast two-hybrid system. Truncation of RBT1 suggests that the domain of RBT1 responsible for binding to RPA32 resides between amino acid 1-120. This region also contains a putative DNA binding domain which needs to be clarified. A GST-RBT1 construct was found to bind *in vitro* translated RPA32. A GFP-RBT1 was transfected into cell line 293 and shown to localize in the nucleus in a pattern similar to, and possibly overlapping with, that of RPA32.

RPA32 was amplified from MCF7 cDNA and cloned, in frame, into pBTM116, a LexA two-hybrid plasmid subsequently referred to as RPA34-pBTM116. This plasmid construct was transformed into yeast strain L40 (MATa trp1 leu2 his3 URA3::(lexAop)8-lacZ LYS2::(lexAop)4-HIS3 lys2 ura3 ade2 gal80 gal4) prior to library transformation. Transcription of the HIS3 reporter gene was found to occur in the absence of protein-protein interaction; this was attributed to potential transactivation function of RPA32. To reduce background, 3-aminotriazole (3-AT), a metabolic inhibitor, was included in media lacking histidine to increase the stringency of the screen. A human osteosarcoma GAL4 cDNA library was amplified according to CLONTECH recommended protocols.

400,000 colonies were screened and 72 colonies were identified which were able to grow on media lacking histidine and containing 25 mM 3-AT. These colonies were restreaked on the same media and

replicated to media containing X-gal. Based on growth rates on SC-histidine (25 mM 3-AT) media and on level of induction of the B-gal reporter gene, positive colonies were classified into three groups, strong, intermediate, and weak interactors. All colonies which demonstrated high levels of induction of the B-gal reporter gene were assayed by PCR using a primer specific to RPA14 and ADC1, a sequence in the terminator region of the library plasmid. Interacting plasmids from several of these colonies were purified and sequenced. Both PCR and sequencing experiments showed that the strongest interactors were representative of RPA32.

A putative positive identified as a RPA32 interacting protein was sequenced and found to have no strong homology to known proteins. This gene, referred to as RBT1, was subcloned from the pACT2 vector into pBTM116 for purposes of mini-screening the two-hybrid library. However, the library could not be screened using RBT1-pBTM116 as bait because of extremely strong transactivation of the yeast reporter genes by itself. This observation suggests that RBT1 may be a transcriptional activator. Although some proteins fortuitously show transcriptional transactivation, its binding to RPA32 supports the notion that RBT1 may not be among such proteins. Experiments were done in attempts to ascertain whether RBT1 functions as a transcriptional activator.

There are several dbEST matches for RBT1 in GenBank. Two representative full length clones have been obtained, DNA was purified, and may be sequenced completely.

Plasmid constructs with truncations at the 3' end of RBT1 have been cloned and transformed into yeast strain L40. ONPG assays shows substantial diminishment